FACTORS AFFECTING PROTEIN DEPOSITION IN RUMINANTS

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SUMMARY

Growth of protein and fat tissues in animals involves increases in cell numbers and cell size to genetically programmed limits but the stage of maturity, which is highly dependent on diet, sets the time scale for relative rates of development of different tissues. Complex control systems in the body influence feed intake, and how nutrients are partitioned between different tissues. Differences between sexes in nutrient partitioning and effects on tissue growth in animals organs or cell cultures, treated with exogenous hormones indicate that the endocrine system is centrally involved in overall control of tissue development. The ratios of circulating nutrients influence the overall endocrine balance and can therefore affect the efficiency of deposition of both protein and fat.

INTRODUCTION

The production of meat, wool and milk by ruminant animals involves the synthesis of protein products in various organs of the body. At one level the process of protein accretion (or secretion) can be regarded simply as the excess of synthesis over degradation of the mixed proteins in the cell, where the relative rates of these two processes, which are each subject to complex control systems, may greatly exceed net synthesis. However, such simplifications ignore the wider aspects of growth and development of various organs and of the body as a whole. Growth of animals also involves synthesis of structural and soft tissues containing protein, fat and water, and changes in the structure and function of these tissues are needed throughout carcass development. To enable their modification, tissues are constantly degraded and the products re-used for synthesis of new tissues. This turnover process involves inevitable energy (ATP) expenditure and wastage of the building monomers, even in mature animals. Different organs and tissues respond differently to nutritional and physiological status, e.g. in maintenance fed animals in energy balance, fat may be mobilized and protein synthesised producing changes in body composition; keratin synthesis and wool growth continue even in grossly undernourished sheep that are rapidly losing weight.

In this review, factors involved in protein accretion and its control at a whole body, individual organ and at a cellular level will be considered.

Tissue growth and body composition

The deposition of protein is one facet of the deposition, growth and differentiation of body tissues. Black (1983) has summarized the factors involved in cellular development of growing tissues which briefly are as follows. Tissue growth involves increases in cell numbers and in cell size and the deposition of fat, protein, cartilage etc.

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Maximum cell numbers and cell size are probably determined genetically (Baldwin and Black 1979). Early growth of organs involves increases in cell size as well as numbers, but division ceases before maximum organ size is attained, and thereafter further growth is by increase in the size of individual cells. For example, Johns and Berger (1976) found that cell numbers in gastrocnemius muscle of lambs increased until the lambs were 35 kg. Numbers of cells were virtually constant between 35 and 45 kg and further growth was due to increases in cell size. In adult animals, changes in organ size, both decreases (with undernutrition, disease etc.) and increases in size are almost entirely due to alterations in cell size - possible exceptions being gut mucosa and skin.

Most of the variation in body composition between genotypes appears to be associated with the rates of change in numbers and sizes of different cells in muscle and adipose tissue, and the timing of these changes with approaching maturity. In an analysis of results from a study by Moulton et al. (1921, 1922) of growth in steers fed a well-balanced diet, Koch et al. (1979) concluded that the level of intake sets the scale for physiological age or stage of maturity which in turn determines how nutrients are partitioned for synthesis into protein and fat. In contrast to the majority of other studies, they found that rate of fat deposition was linear with time from 5 months to 4 years of age, after an initial lag of about 6 weeks, with the rate being lower for steers on restricted feed intakes. The rate of deposition of metabolically active tissues (which were mostly protein, i.e. 3.04 ± SEM 0.02 kg nitrogen, and water), decreased exponentially with time, and approached the same asymptotic value for all planes of nutrition (see Fig. 1).

![Diagram of tissue mass over time for high, medium, and low planes.](image)

Fig 1

The development of tissue components (i.e. metabolically active tissue, largely protein plus water) or fat over time in three groups of steers (Hereford x Shorthorn) fed the same good quality diet at three levels (i.e. 83, 47 or 33 MJ/d) from age 3 months to 4 years (from Koch et al. 1979)
At lower body weight, irrespective of age, animals contain mostly lean tissue with little fat. As body weight increases the percentage of fat also increases.

Elsey (1976) reviewed the results of 179 experiments designed to test the effects of gross nutritional manipulations of both energy and protein on body composition and concluded that "the composition of the fat-free mass is largely immutable". Searle and Griffiths (1983) also concluded that the amounts of protein in the fat-free empty body weight (EBW) of growing sheep were independent of nutritional treatments imposed during growth, and, as fat-free, EBW increased from 5 to 40 kg, the percentage of protein increased from 17.1 to 21.0 (asymptotic value = 21.5%). In contrast, when protein content is expressed as a percentage of EBW (including fat), there is a linear decrease in protein percentage with approaching maturity which differs between male animals and females (or castrates) at different liveweight or stages of maturity (Thompson et al. 1985). Apparent differences in the percentage of protein in the EBW between strains of sheep (selected for high or low weaning weights) at different body weights were removed when the results were compared at the same stage of maturity (see Fig. 2).

![Fig. 2](image_url) The protein content in the body of Merino sheep at different body weights or different stages of maturity as affected by genetic selection for growth, or sex of animal. Two of the strains of sheep examined were obtained by selection for high (weight-plus) or low (weight-minus) weaning weight, and the third was a randomly bred control (from Thompson 1985)
Measurement of Protein accretion degradation during growth

Changes in protein content of the body have commonly been studied by the nitrogen (N) balance technique, or by slaughtering animals representative of treatment groups at various times and determining changes in carcass protein content. These techniques provide information on the net effect of differing rates of synthesis or breakdown of protein in the whole-body but do not estimate synthesis and degradation separately. For this purpose, measurements of whole-body turnover have been made using labelled amino acids (e.g. $^{15}$N-glycine, $^{13}$C-leucine, or $^{3}$H-leucine) in conjunction with N balance measurements (see Waterlow et al. 1978).

Measurements of whole-body turnover indicate that both synthesis and degradation continue, even in animals in negative N balance (see Fig. 3).

![Fig 3](image)

The relationship between protein synthesis and protein deposition in the body of pigs, wallabies and human beings. Data are from Reeds et al. (1985) and R.G. White, I.D. Hume and J.V. Nolan, (unpublished)

A one unit increase in protein deposition is accompanied by about a two unit increase in protein synthesis, although the precise relationship varies, particularly with respect to the protein:energy ratio in the absorbed nutrients. An increase in growth resulting from additional absorbed protein produces a greater increase in protein synthesis than a similar increase in growth brought about by extra non-protein energy (Reeds and Fuller 1983).

These whole-body techniques have been extensively used and evaluated in studies of protein metabolism in human subjects but there are few studies of the suitability of these methods for use with ruminants. Recent studies by Cronje (1987) indicated that $^{35}$S-methionine, $^{3}$H-leucine and $^{15}$N-lysine when used simultaneously gave similar estimates of whole-body protein turnover in lambs on a low protein roughage diet, but inconsistent results in sheep given higher levels of protein supplementation (see Fig. 4). The use of at least two markers simultaneously was recommended. In the lambs fed the low protein basal diet values for whole-body turnover in sheep were lower than the accepted inter-species mean (16 g protein/W, see Buttery 1984) which was only attained when the basal diet was supplemented with 200 g/d of a
pelleted protein supplement (providing 380 g crude protein/kg) and formulated to provide amino acids for absorption post-ruminally.

![Graph showing protein turnover](image)

**FIG. 4** Whole body protein turnover estimated by intravenous infusion of $[^{35}S]$-methionine, $[^{3}H]$-leucine and $[^{14}C]$-lysine in sheep fed a basal diet of wheat straw, urea and minerals and a pelleted bypass protein supplement at 0, 40, 120 and 200 g/d

During periods of underfeeding, net degradation of protein occurs in muscle with release of amino acids into the circulation, while uptake of amino acids occurs in other organs such as the mammary gland and the liver. This limits the usefulness of whole-body protein turnover estimates and for this reason, many laboratories have developed methods for estimating the extraction and release of peptides and amino acids across individual organs such as the hind limb and mammary gland. These methods enable the effects of nutritional and hormonal treatments on protein turnover in individual organs to be studied in detail (e.g. McDowell et al. 1984; Jois et al. 1986).

**Intracellular protein synthesis**

This process is now quite well understood (see Pain and Clemens 1980). Briefly, the synthesis of various cellular proteins in cells involves the assembly of amino acids into polypeptide chains according to instructions provided by RNA *codons* transcribed from segments (genes) of the master blueprint, i.e. the nuclear DNA. Three kinds of RNA and a large number of proteins are involved in the process of translation. Messenger-RNA (*m-RNA*) is bound to ribosomes and its nucleotide sequence determines the order in which amino acids are assembled into the polypeptide chain. (The ribosome is an organelle consisting of ribosomal-RNA (*r-RNA*) in combination with about 70 proteins which function as enzymes or determine the structure of other components of protein synthesis.) Transfer-RNA (*t-RNA*) exists in different forms, each one specific for an individual amino acid; it is involved in activating and binding amino acids to the ribosome according to the sequence specified by the messenger-RNA on the ribosome.
The process of translation has three stages:

Initiation - a ribosome and a molecule of specific initiator t-RNA bind to the m-RNA at the start of the encoded sequence whereupon a second t-RNA molecule (and the associated amino acid) can bind and synthesis of the first peptide bond takes place. Two mol ATP are expended per mol amino acid prepared for protein synthesis.

Elongation - the ribosome then moves along the m-RNA decoding the sequence and so producing the appropriate polypeptide chain. Two mol GTP are expended per mol amino acid incorporated.

Termination - at the end of the m-RNA coding sequence, the ribosome and the completed protein chain are released and the ribosome then forms part of a pool of ribosomes awaiting attachment to the same or another molecule of m-RNA.

The rate of synthesis of protein can be regulated either by the number of ribosomes in the cell (which determines the maximum rate), or by the synthetic activity of each ribosome which seems to be controlled by the rate of initiation. Nutritional deficiencies and hormonal levels appear to affect both regulatory processes (see Pain and Clemens 1980). Factors that may affect protein deposition in animals can be studied in cell cultures. At least in Ehrlich ascites tumour cells, disaggregation and reformation of ribosomes occurs in response to essential amino acid depletion, and repletion in the culture medium within 10 minutes. Culturing without glucose also results in disaggregation of ribosomes although the effect occurs more slowly (Van Venrooij et al. 1972). Protein balance is affected by leucine concentration which stimulates synthesis (and inhibits breakdown) (Goldberg et al. 1980). Thus the availability of glucose and essential amino acids may affect rates of protein synthesis in cells. The assimilation of these and other nutrients is also subject to hormonal control (Young 1980). Hormones that stimulate protein synthesis include insulin (which also stimulates uptake of glucose and amino acids by cells), and the androgens. Thyroid hormones and growth hormone also markedly stimulate protein synthesis (and breakdown) and when either is deficient, normal rates of cell growth cannot occur. Concentrations of hormones depend on the age of the animal, and are higher in animals with a higher genetic potential for growth (Verde and Trenkle, 1987) but the interrelationships between age, hormonal status and nutrient supply are still poorly understood (Young 1980).

**Intracellular protein degradation**

Whilst a considerable amount is known about the mechanism and control of protein synthesis in cells, less is known of the processes of degradation. Three separate processes of intracellular peptide-bond cleavage are recognised (a) a co-translational proteolytic mechanism located in the rough endoplasmic reticulum, which removes 'signal' peptides from secretory proteins (Blobel and Dobberstein, 1975), (b) a rapid process that degrades a large proportion of newly-synthesised protein (e.g. a large proportion of collagen is destroyed within fibroblasts by a co-translational or immediate post-translational process) and (c) a post-translational process which is subject to physiological regulation and which may be affected by the properties of the protein (e.g. susceptibility to proteolytic digestion). There is evidence

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that mammalian cells can selectively degrade abnormal proteins. Ballard (1977) proposed two independent mechanisms of protein degradation - direct proteolytic breakdown, perhaps membrane-linked process in which enzymes are first inactivated and then degraded; and autophagy, a process by which cell contents are enclosed by membranes to form vacuoles which fuse with lysosomes in which the proteins are degraded.

Autophagic activity in hepatocytes, gauged by the number of autophagic vacuoles observed by electron microscopy, is rapidly reduced by insulin administration (Pfeifer et al., 1978) and increased by glucagon (Ashford and Porter, 1962). Inhibitors of lysosome function reduce protein breakdown by 15-40% and can reduce muscle atrophy in organ culture (Goldberg et al. 1980). Depression of ATP levels by administration of cyanide or dinitrophenol reduces protein degradation (Simpson, 1953), suggesting a means whereby protein deposition may be controlled by availability of substrates supplying energy.

**Tissue of Protein deposition**

The interrelationships between rates of protein synthesis and degradation are maintained by complex control systems with neural and hormonal facets (Bassett, 1978), which, furthermore, may be genetically determined. In a recent study (Verde and Trenkle, 1987), 4 steers with high growth potential (Simmental x Brown Swiss) were compared with 4 steers with lower growth potential (Angus, Angus x Hereford), ad libitum feed intake and rate of gain were respectively 51% and 45% higher in the Simmental cross steers and these steers had higher mean concentrations of growth hormone, insulin, thyroxine and cortisol. The differences were unrelated to age or physiological maturity. The average secretion rate of growth hormone in growing steers has been found to be correlated positively with growth of lean tissue and negatively to gain of fat (Tenkle and Topel 1978).

A recent finding that must profoundly alter current understanding of the effects of growth hormone is the effect of exogenous growth hormone on the exchange of peptide-associated plasma amino acids across hind limb and mammary tissues whilst there was little effect on exchanges of plasma free amino acids (Jois et al. 1985). These results not only draw attention to an important role of plasma peptides as transporters of amino acids in the body, and, in addition, to possible specific roles in overall control of protein metabolism in the body.

Millward et al. (1981) have observed that growth appears to occur in skeletal muscle at times, by different mechanisms. On the one hand, degradation rates are relatively high in rapidly growing muscle of young as compared with adult animals, implying accompanying even higher rates of synthesis. These workers consider that the high rate of degradation may not be directly associated with growth, but rather with myofibrillar remodelling and enlargement (Millward et al., 1975). On the other hand, rapid growth can at times be achieved at a lower rate of protein synthesis, with a correspondingly lower rate of degradation. An example of this process is the increased growth of female rats (Vernon and Buttery, 1976) and sheep (Sinnet-Smith, 1983) given the anabolic steroid, trenbolone acetate which reduced degradation rate by up to 30% relative to untreated controls. The mode of action of this androgenic drug is still unclear, but its effect on degradation rate is opposite to that of testosterone which increases the fractional synthetic rate of muscle whilst also promoting growth (Martinez et al., 1984).
The complex and interactive nature of processes controlling protein deposition and growth means that it is extremely difficult to make accurate quantitative predictions of the likely production responses to dietary or other manipulations. Ruminant animals on fibrous diets with low nitrogen content often have low feed conversion efficiencies, and are responsive to dietary manipulations aimed first at maximising rumen fermentative digestion, and second at providing additional glucose for intestinal absorption, or providing additional precursors to promote gluconeogenesis (Leng and Preston, 1976). In practice, protein supplements are often particularly effective (Lee et al., 1987). These provide bypass amino acids (essential and glucogenic), which increase efficiency of utilisation of total nutrients through improvements in the circulating nutrient profile, and enhance feed intake, probably through a combination of effects on body control systems. They may also improve microbial growth efficiency in the rumen increasing the amounts and favourably altering the ratios of fermentation end-products. They are also commonly provided when animals have a disposition to produce proteinacious tissue which favours a more efficient gain in liveweight per unit of metabolizable energy.

During periods of feed restriction, there is release of free amino acids from muscle and uptake by the liver. This process is probably affected by the glucocorticoids, which have a complementary action, inhibiting protein synthesis and promoting protein breakdown in muscle and stimulating gluconeogenesis from amino acids in the liver (Goldberg et al. 1980). Protein:energy (glucose) interrelationships

The rate of deposition of protein in the body of ruminants is responsive to the level and balance of absorbed amino acids and of the major energy-yielding substrates i.e. VFA, glucose and lipid. Ruminants on roughage diets absorb negligible amounts of glucose and are almost entirely dependent on gluconeogenesis, whereas ruminants given grain-based diets, like simple-stomached animals, may absorb glucose from the small intestine. For ruminants, there is potential for manipulating the availability of nutrients for growth by altering the nature of the fermentative digestion (by nutrient supplementation of the diet or use of chemical agents) or by supplying bypass nutrients, or both. However, in practice, it is difficult to identify the primary limiting nutrients in order to determine how to correct any imbalance. The requirement for any one nutrient depends on the availability of and metabolic needs for other nutrients.

Imbalances in the profile of absorbed nutrients reduce growth and feed conversion efficiency. If essential amino acids are not absorbed in sufficient quantities to meet the losses in the body associated with turnover processes, plus the amounts needed for protein accretion, then an additional supply of digestible amino acids to the small intestine will be needed to improve net protein synthesis and growth. However, the requirements for amino acids could be altered in a variety of ways, e.g. by use of exogenous hormones that affect protein turnover processes, or by increasing the supply of non-amino acid glucogenic precursors such as propionate, or by altering the supply of materials involved in lipid deposition. Biochemical interrelationships between amino acid, glucose and lipid metabolism are discussed in detail by Preston and Leng (1986) and Nolan et al. (1986). However, the possible effects of variations in the profile of circulating nutrients (glucose, amino acids, free fatty acids) on overall control systems affecting feed intake regulation and efficiency of utilization of absorbed nutrients are largely unknown.
REFERENCES